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DEMANDS DURING MAZE LEARNING IN LIMBIC EPILEPTIC RATS: SELECTIVE DAMAGE IN THE THALAMUS?[1]

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Summary.—A qualitatively evident enhancement of chromolytic neurons within the lateral posterior thalamus of rats in which limbic seizures had been induced by lithium and pilocarpine and who were later trained for spatial memory was assessed quantitatively. The significant increase in the numbers of chromolytic neurons and the decrease in the numbers of normal neurons for these rats compared to the reference brains suggested these morphological changes were recent. The hypothesis that excessive stimulation of the lateral posterior nucleus by daily training in a radial maze may have facilitated the necrosis was supported by the inverse relationship between a linear combination of the numbers of normal neurons and oligodendroglia and the rate of learning during the earlier but not the later sessions. An implication for iatrogenic effects from rehabilitation of humans following brain injury was suggested.

Neuroplasticity, defined as the capacity to alter quickly and permanently neuronal activity in response to the neuroelectrical representation of experience, has allowed the mammal to adapt to its environment. One consequence of this microstructural responsiveness to brief experiential representations is enhanced vulnerability of some neuronal soma to necrosis or apoptosis. For example, Sapolsky (1994) has shown that pre-exposure to physiological elevations of glucocorticoids increases the vulnerability of hippocampal cells to death during relatively routine stimulation and that this vulnerability may be associated with subtle individual differences.

We (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993) have shown that chronic limbic epilepsy, induced by a single systemic injection of lithium (3 mEq/kg) followed four hours later by a single, systemic injection of pilocarpine (30 mg/kg), evokes a permanent electrical lability. This lability has been inferred by the persistent overt displays of limbic motor automatisms (about once/day) that are indistinguishable from the rearing, forelimb clonus, and falling evoked by the direct electrical stimulation of limbic structures during kindling. However, unlike kindling, which requires several hundreds of direct stimulations before spontaneous, i.e., not elicited by direct stimulation, overt limbic seizures emerge, the rats which have received the lithium/pilocarpine treatment become chronically epileptic after this single episode.

Histomorphological examination of the brains of rats in which limbic

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seizures have been induced by lithium/pilocarpine reveals consistent damage within primarily thalamic and subcortical telencephalic nuclei (Bureau, Peredery, & Persinger, 1994). Although different nuclei exhibit both estimates and direct measures of cell loss that range from less than 5% to more than 95%, the magnitude of damage within a particular nuclear group remains consistent. The continuation of micromorphological changes for weeks after the single systemic injection of lithium/pilocarpine has been shown by the appearance of crystalline, calcium-containing material within those brain nuclei that initially displayed the greatest amount of cell loss (Lafreniere, Peredery, & Persinger, 1992).

We have been pursuing the hypothesis that during periods of enhanced electrical and chemical vulnerability within specific neuronal ensembles *normal* electrical activity associated with the normal representation of experience would be sufficient to evoke the death of neurons that have been marginally maintained. Our unpublished studies have suggested that the suprageniculate nucleus of the thalamus, which is involved with auditory-visceral processing and acoustic startle, was damaged if the rats were exposed to frequent, sharp sonic stimuli (sufficient to evoke the startle reflex in the normal rat) during the first 24 hr. following the induction of the seizure. During extended, microscopic examination (1000 ×) of the brains of rats whose behavioral histories and general brain damage were reported elsewhere (Bureau, *et al.*, 1994), we noted an unusual morphological profile within the lateral posterior thalamus that was not evident in several comparator regions that included the mediodorsal thalamus, CA1 section of the dorsal and ventral hippocampus, insular cortices or forelimb cortices. The anomaly was a conspicuous increase in the numbers of glial cells and oddly staining neurons.

If this anomaly was a consequence of recent death due to the rats' daily requirements to discriminate and remember a visuospatial, radial maze task, then the following predictions should be supported. First, the numbers of neurons discernable within the lateral posterior thalamus after eight days of daily training should be positively correlated with the rats' performances during the first few days of maze acquisition (or fewer neurons should be associated with poorer performance because the consequence of the experiential representation would have destroyed those neurons). With repeated exposure to the maze environment (later sessions), the consequences of repetition and contributions from other, less affected portions of the brain would be expected to compensate for the necrosis within the lateral posterior thalamus. Second, evidence of the recency of morphological alteration such as necrosis should be evident by the greater numbers of chromolytic neurons relative to normal neurons and by the increased numbers of astroglia cells relative to numbers in the brains of rats from the reference group.

Method

Source of Data

Re-analysis of data from the brains of chronic epileptic rats that were tested in the Olton, 8-arm maze (Harrigan, Peredery, & Persinger, 1991) and our automated four-arm, lever-pressing maze (Persinger, Bureau, & Peredery, 1995) suggested a quantitative relationship between the amount of damage in the lateral posterior group and the accuracy of "memory" during the stages of learning that would depend most upon spatial integration. Serial multiple regression for the learning proficiency of each of the eight days of testing indicated that different brain structures may be more essential for different stages of acquisition than previously suspected. While the lateral posterior nucleus was important in the early stages of learning, the suprageniculate (perhaps associated with the click of the rewarding magazine) and the forelimb portion of the cerebral cortex (associated with the increased requirement for manipulation of the reinforcement lever) were more strongly correlated with success during the later stages of acquiring the automated maze task.

Sample

To test this hypothesis a total of 20, approximately 120 day to 150 day old, male Wistar albino rats were selected from the Behavioral Neuroscience Brain Library. Each of approximately 200 rat brains are maintained as 50 to 100 coronal, 10-micrometer paraffin-embedded sections that were obtained by microtome between the posterior commissure and anterior commissure. All rats had been approximately 90 days of age when they had been injected subcutaneously with either 2 mEq/kg or 3 mEq/kg of lithium and then 4 or 24 hr. later with either 20 mg/kg or 30 mg/kg of pilocarpine. The brains had been removed within one week of the termination of the behavioral training in a maze, fixed in ethanol-formalin-acetic acid, and processed. Every third section had been stained. The rats' behavioral data were recovered from computer disc. The numbers of trials completed per 30-min. sessions during radial (four-arm) maze learning for the first four sessions and for the second four sessions were extracted. The means and standard deviations for these values were 4.9 (5.5) and 14.1 (16.2). For this study, the numbers of normal neurons, chromolytic neurons (defined by displaced nuclei and dispersed Nissl substance), astroglia nuclei, oligodendroglia nuclei and microglia per field were counted at 1000 × light microscopy within three fields from the left and right sides of the medial and the lateral division of the lateral posterior nucleus of the thalamus. Only neurons whose nuclei were visible were counted.

Analysis

These values were compared by one-way analysis of variance to three

brains from rats that had not received any treatment but had been single-caged for about two months before decapitation. To verify any reciprocal relationship between the observation of normal and chromolytic neurons, repeated-measure multivariate analysis of variance was also performed as a function of group (seizure vs reference) and the numbers of normal vs chromolytic neurons within the lateral and medial components of the lateral posterior nucleus. To accommodate for individual variability, the ratio of the square root of the numbers of the mean numbers of completions per day for the first four days divided by the square root of the average successful completions for all eight sessions and the ratio of the square root of the mean numbers of successful trials per day for the second four days divided by the square root of the average for all sessions were obtained for each rat. Multiple regression analyses for these two dependent variables as well as the square roots of the numbers of total trials per day during the entire 8-day test period were completed as a function of the five cytological measures for the lateral and medial components of the lateral posterior nucleus. Because the sample was limited in size, only the first two variables were allowed to enter the (stepwise) equation. All analyses involved SPSS software on a Vax 4000 computer.

Results

The cytometric results are shown in Table 1 and are expressed as numbers of cells per mm-squared. The quantitative measurements verified the qualitative observations. The rats in which seizures had been induced and which had received daily exposures to learning within the radial maze displayed significantly more chromolytic neurons, fewer normal neurons, and more astroglia. A three-way analysis of variance with two levels repeated (normal and chromolytic nucleus vs lateral or medial portion of lateral posterior nucleus) as a function of rats with a seizure history or no history yielded a statistically significant interaction ($F_{1,20} = 22.69$, $p < .001$; eta = 0.72) between the morphology of the cell and the history of the group. There were no statistically significant main effects between groups ($F_{1,20} = 0.22$, $p > .05$), differences between the numbers of normal cells and chromolytic cells ($F_{1,20} < 1.00$, $p > .05$), or higher order interactions.

The multiple regressions indicated that the cytological measurements significantly predicted the relative numbers of successful trials per day (learning) during the first half of the eight, successive daily trials but neither the latter half of the learning period nor the total number of successful trials. The first two variables to enter the equation, whose multiple R was 0.71 ($F_{2,16} = 8.01$, $p < .01$; adjusted $r^2 = 44\%$), were the numbers of oliodendroglia (beta = 0.66) and the number of normal neurons (beta = 0.54) within the lateral group only. [It may be of interest that, were three variables allowed to enter the equation, the third was the numbers of astroglia (beta = −0.42).]

TABLE 1
MEANS AND STANDARD DEVIATIONS FOR NUMBERS OF CELL TYPES PER MM-SQUARED WITHIN LATERAL AND MEDIAL PORTIONS OF THE LATERAL POSTERIOR THALAMIC NUCLEUS

Source	Lateral Portion				Medial Portion			
	Normal		Seizure		Normal		Seizure	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Normal neurons	362	138	193*	90	360	101	163*	69
Chromolytic Nucleus	169	165	439*	129	252	134	448*	135
Oligodendroglia	102	78	108	44	152	53	133	60
Astroglia	249	75	406*	112	223	45	436*	161
Microglia	30	24	20	25	25	40	34	38

*Seizure group significantly ($p < .05$) different from controls.

To resolve the issue of recency of the cytological differences, the brains in which G-factor was present ($n = 6$) within the lateral posterior nucleus were differentiated from brains ($n = 14$) that did not contain this substance. The accumulation of this factor, which is composed of Nissl-membrane debris and complexes of calcium and mucopolysacharides, requires at least 20 to 30 days after the initiation of the seizures (Lafreniere, Peredery, & Persinger, 1992). There was no statistically significant difference ($F_{1,18} = 1.07$, $p > .05$) between the mean numbers of successful trials per day during the first half of the experiment for rats that displayed G-factor ($M = 3.0$ and $SD = 0.4$) or those that did not ($M = 5.8$ and $SD = 6.2$). Given the heterogeneity of variance, a Kruskal-Wallis nonparametric test was performed but was also not significant statistically ($\chi^2 = 0.33$).

DISCUSSION

These results suggest those rats that exhibited the fewest successful trials (poorer learning) during the first sessions of maze learning displayed more evidence of neuronal dysfunction as defined by fewer observable nuclei of oligodendroglia (the primary source of myelin) and more astroglia (a common correlate of reactive gliosis) within the lateral posterior thalamus. If we assume that the pattern of cell micromorphology is correlated with behavior, then the results suggest that this morphology in the lateral thalamus was most associated with the early stages of learning. That the cytological changes were recent and were within the time frame of the maze experience before the brains were fixed is indicated by the absence of differences between the acquisition scores for rats for which brains did or did not contain a morphological signature (G-factor) or earlier damage.

Although correlation only reflects association, the type of neuromorphology strongly suggests that the exposure to the maze and the demands upon the lateral posterior thalamus by this exposure may have evoked excitotoxic stimulation. Consequently, more neurons became chromolytic as in-

dicated by their statistically significant increase in incidence while the total numbers of neurons (normal plus chromolytic) did not differ between rats with histories of limbic seizures or normal backgrounds. The recency of the chromolysis (during the maze training) is more likely since these manifestations are usually not maintained and only one week had elapsed before the rats were decapitated but up to two months had elapsed since the induction of the seizures. The fact that about one-third of the neurons within the reference brains exhibited indications of Nissl disturbance or eccentric nuclei while the rats with histories of seizures and maze training exhibited these indicators in more than two-thirds of the neurons (but no loss of total numbers of neurons) suggests that the maze-training may have exacerbated a normal neuronal process.

These results suggest that vulnerable aggregates of neurons which mediate specific functions during learning may undergo necrosis if these functions exceed the threshold for accommodation. The doubling of astroglia cells in the rats with histories of seizures and recent maze training compared to the numbers of such cells in the reference brains despite no group differences in the total numbers of neurons is consistent with recent reactive gliosis to alterations in local blood-brain barriers. This conclusion would also be compatible with the observation of the increased proportion of neuronal soma that were visibly chromolytic.

The caveat to this statement is the possible confounding effect of handling, maintained food deprivation, and other correlative operations of maze training. However, if this qualitatively conspicuous effect is generalizable to rehabilitative settings for human beings, programs that emphasize strategies of maximum daily effort for patients who are recovering from brain trauma associated with subclinical limbic seizures might reevaluate the subtle consequences upon synaptic reorganization and changes in personality (Persinger, 1995). Stated alternatively, some long-term consequences of alterations in behavior and personality following "occupational," "physical," or "cognitive" therapies that emphasize forced, repetitive tasks which load the same neural substrates may be created by the direct consequences of the rehabilitation process.

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